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# Insulin-like growth factor-1 polymorphism and its association with economic traits in Kadaknath and its crossbred birds

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ABSTRACT

Insulin-like growth factor-1 (IGF-1) gene polymorphism has been investigated by PCR-RFLP method on 80 unrelated female birds each of Kadaknath and its crossbred selected randomly from Department of Poultry Science of the University. The 3 different RFLP patterns revealed restriction fragment of 621, 364 and 257 bp sizes in Kadaknath and Kadaknath crossbred birds. This indicated presence of 2 restriction sites at 621 bp (site A) and 364, 257 bp (site B) position in 621bp amplicon. Genotypic frequencies in Kadaknath and Kadaknath crossbred birds were found to be 0.41 and 0.35 for AA genotype, 0.37 and 0.38 for AB genotype and 0.21 and 0.27 for BB genotype, respectively, whereas the allelic frequencies in Kadaknath and Kadaknath crossbred birds were 0.60 and 0.54 for A allele and 0.40 and 0.46 for B allele respectively. The Kadaknath population was in Hardy Weinberg equilibrium, however Kadaknath crossbred population under study was not in Hardy Weinberg equilibrium. The differences between least squares means of various genotypes for age at sexual maturity and adult body weight at 20, 40 and 52 weeks were nonsignificant (P>0.05). Genotype BB was superior for body weight at 20, 40 and 52 weeks in Kadaknath and Kadaknath crossbred birds. Significant breed × genotype interaction was found for egg production at 40 weeks of age and higher egg production was recorded for AA genotype in Kadaknath and AB genotype in Kadaknath crossbred birds. Genotype AA was superior for egg weight at 40 and 52 weeks in Kadaknath and Kadaknath crossbred birds.

**Key words**: Economic traits, Insulin-like growth factor-1, *IGF-1* gene, Kadaknath, Kadaknath crossbred birds, PCR-RFLP

Egg production in India is increasing at the rate of 6 to 8% and broiler production is increasing at the rate of 12 to 15%. At present India is third largest producer of eggs (next to China and USA) and fifth major producer of broiler in the world (next to USA, China, Brazil and Mexico). Insulin like growth factor-1 (IGF-1) gene has 621 base pair and consists of 4 exon and 3 introns spanning over more than 50 kb on chromosome 1 (Bian *et al.* 2008). IGF-1 influences growth rate, body composition and lipid metabolism in poultry (Beccavin *et al.* 2001). Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis is a useful technique for screening of sequence variation resulted due to polymorphic restriction enzyme (RE) sites. RFLP have certain properties like expressed in co-dominant fashion, multi-allelic, unaffected by the environment, stably inherited, and generally expected to be devoid of pleiotropic effects on economic characters (Hillel *et al.* 1992). Due to importance of *IGF-1* gene in regulating many physiological and metabolic processes, the *IGF-1* gene was chosen as a promising candidate gene when searching for genetic variants associated with production traits. Chicken *IGF-1* gene is highly polymorphic and was found associated with various economic traits in birds (Li *et al.* 2009). The present study was to determine association between various polymorphic variants of IGF-1 gene and economic traits using PCR-RFLP method in Kadaknath and Kadaknath crossbred birds.

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## MATERIALS AND METHODS

*Resource populations and DNA isolation:* Blood samples from 80 unrelated female birds each of Kadaknath and Kadaknath crossbred birds (developed by crossing and backcrossing of purebred Kadaknath male with black plumage dual type colour birds at AICRP on Poultry, Jabalpur) selected randomly from All India Coordinated Research Project (AICRP) on Poultry Breeding, College of Veterinary Science and Animal Husbandry, Jabalpur. Blood sample (2 ml) of each bird was collected aseptically from wing vein in sterile EDTA coated vacutte tubes. After proper labeling, the samples were transported to the laboratory in an icebox for further analysis. The blood samples were kept

at –20°C till further analysis. Genomic DNA was extracted as per John *et al.* (1991) with slight modifications. The purity and quantity of the extracted DNA was checked by Nanodrop spectrophotometer and quality of DNA was assessed on 0.8% horizontal submarine agarose gel electrophoresis. The primer sequence specific to *Insulin like growth-1* gene was used as described by Nagaraja *et al.* (2000). Exon and intron region from 5' untranslated region of the *IGF-1* gene amplified to a product of 621 bp fragment of the *IGF-1* gene was amplified by PCR using the following forward and reverse primer. (Forward) 5'-GACTATACAGAAAGAACCCAC-3' (Reverse) 5'-TATCACTCAAGTGGCTCAAGT-3'. After checking the amplification of PCR product through 2.0% agarose gel. The 5 µl of amplified PCR product mixed with 1 µl of 6x gel loading dye (bromophenol blue) and loaded along with 100 bp DNA ladder as a molecular size marker in a separate lane. The amplified PCR product of each sample was digested using *Providencia stuarti (Pst1)* restriction endonucleases (RE). PCR product of each sample 12.5 µl was digested with 1.5 µl *Pst1* in manufacturers recommended assay buffers in a final reaction volume of 20 µl. The reaction mixture was incubated at 37°C for overnight digestion in water bath. The digested PCR products were analysed by 2.0% agarose gel electrophoresis and ethidium bromide staining, and were classified into 3 different RFLP pattern revealed restriction fragment of 621 (AA), 364 and 257 (BB) bp sizes (homozygous) and 621, 364 and 257 (AB) bp sizes (heterozygous) in Kadaknath and Kadaknath crossbred birds. Genotype frequencies, gene frequencies and genetic equilibrium at different loci were estimated using software POPGENE 32 version 1.32, the user-friendly software for population genetic analysis (Yeh *et al.* 1999) and the association of various polymorphic variants of IGF-1 gene with economic traits (age at sexual maturity, adult body weight at 20, 40 and 52 weeks, egg production at 40 and 52 weeks and egg weight 40 and 52 weeks) were analyzed by mixed model least squares and maximum likelihood computer program PC-2 (Harvey 1990).

$$Y_{ijk} = \mu + a_i + B_j + (aB)_{ij} + e_{ijk}$$

Where,

$Y_{ijk}$ is the economic trait of $k^{th}$ bird of the $j^{th}$ breed for $i^{th}$ genotype; µ, overall mean; $a_i$, set of random cross classified effects due to genotypes; $B_j$, set of fixed effect due to breeds; $(aB)_{ij}$, interaction between $i^{th}$ genotype with $j^{th}$ breed; and $e_{ijk}$, random error.

## RESULTS AND DISCUSSION

PCR was performed with selected primer for all the samples and the PCR product was run in 2% agarose gel. Present study revealed amplified PCR product of 621 bp size in Kadaknath and Kadaknath crossbred birds. PCR product of the 621 bp was also reported by Li *et al.* (2008) in Wenchang chickens and Li *et al.* (2009) in 3 canopy chickens. PCR product on digestion with *Pst1* restriction endonuclease revealed restriction fragments of 621, 364 and 257 bp sizes in Kadaknath and Kadaknath crossbred birds. This indicated

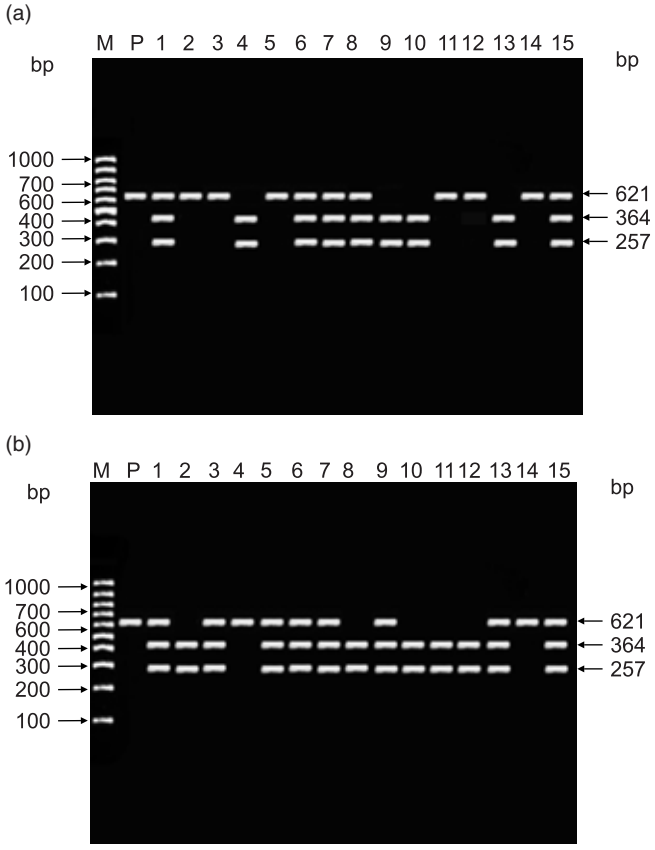


Fig. 1. (a) PCR-RFLP pattern of *IGF-1* gene digested with *Pst*1 enzyme (a) Kadaknath birds M, 100bp DNA ladder; P, PCR product;lane 4, 14, AA genotype (621bp);lane 1,3,5–7,9,13,15, AB genotype (621,364,257bp); lane 2,8,12, BB genotype (364,257bp). (b) Kadaknath crossbred birds. M, 100bp DNA ladder; P, PCR product;lane 2,3,5,11,12,14, AA genotype (621bp);lane 1,6–8,15, AB genotype (621,364,257bp);lane 4,9,10,13, BB genotype (364,257bp).

Table 1. Frequencies of genotypes and alleles at IGF-1 locus

| Breed | Genotype | | | Allele | | Chi-square value |
|---|---|---|---|---|---|---|
| | AA | AB | BB | A | B | |
| Kadaknath | 0.41 (33) | 0.38 (30) | 0.21 (17) | 0.60 | 0.40 | $1.99^{NS}$ |
| Kadaknath crossbred | 0.35 (28) | 0.39 (31) | 0.26 (21) | 0.54 | 0.46 | 4.97* |

NS, Nonsignificant; *significant (P<0.05) and figure in parenthesis show number of birds in each genotype.

presence of 2 restriction sites, 1 at 621 bp, (site A) and another at 364 and 257 bp (site B) position in 621bp amplicon. These restriction fragments showed 3 different RFLP patterns. The RFLP pattern-I consisted of fragment 621bp size symbolized as AA genotype, RFLP-II consisted of 364 and 257 bp sizes symbolized as BB genotype and RFLP pattern-III consisted of fragment of 621, 364 and 257 bp symbolized as AB genotype (Fig. 1a, b).

The RFLP fragments and patterns of genotypes obtained in this study are in agreement with RFLP fragment and patterns obtained by Li *et al.* (2008) and Li *et al.* (2009). Genotypic frequencies in Kadaknath and Kadaknath crossbred birds were 0.41 and 0.35 for AA genotype, 0.38 and 0.39 for AB genotype and 0.21 and 0.26 for BB genotype respectively, whereas the allelic frequencies in Kadaknath and Kadaknath crossbred birds were 0.60 and 0.54 for A allele and 0.40 and 0.46 for B allele (Table 1). Significant Chi-square value in Kadaknath crossbred birds indicated that population was not in equilibrium. The probable reason might be that 2 different parental populations were used for generating the crossbreds, might be significantly different from each other in respect to gene frequency. Nonsignificant Chi-square value was obtained in Kadaknath breed of chicken indicating that population was in equilibrium.

Present study revealed higher genotype frequency of AA genotype in Kadaknath and AB genotype in Kadaknath crossbreds, whereas the higher gene frequency was observed for 'A' allele in Kadaknath and Kadaknath crossbred birds than 'B' allele (Table 1). A similar result for genotype frequency of AA genotype was also reported by Enyati and Rahimi-Mianji (2009) in Mazandaran native fowls and for population equilibrium was reported by Thakur *et al.* (2009) in Kadaknath breed of poultry.

*Association of insulin like growth factor-1 gene polymorphic variants with economic traits:* The results of least squares analysis of variance (Table 2) showed highly significant (P<0.01) effect of breed, whereas the effect of genotype and breed × genotype interaction were nonsignificant for age at sexual maturity. Highly significant (P<0.01) differences were observed between least squares means in 2 breeds for age at sexual maturity, adult body weight at 20, 40 and 52 weeks of age, egg production and egg weight at 40 and 52 weeks, however the least squares means for various genotypes within breeds were nonsignificant.

The results reported in this study for age at sexual maturity are in agreement with results reported by Kaur *et al.* (2008) in meat type chicken stain PB-2 and Singh *et al.* (2008) in RIR, WLH and their crosses. Similar findings for mean body weight at different ages, egg production and egg weight were reported by Shafi *et al.* (2008) in Bantom, Bantomized White Leghorn and White Leghorn breed.

The least squares means for various genotypes were nonsignificant for age at sexual maturity, adult body weight

Table 2. Least squares means for various economics traits for breeds, genotypes and breed × genotype interactions

| Factors | Age at sexual maturity (days) | Adult body weight at 20 weeks (g) | Adult body weight at 40 weeks (g) | Adult body weight at 52 weeks (g) | Egg production at 40 week (numbers) | Egg production at 52 weeks (numbers) | Egg weight at 40 weeks (g) | Egg weight at 52 weeks (g) |
|---|---|---|---|---|---|---|---|---|
| Breed | ** | ** | ** | ** | ** | ** | ** | ** |
| Kadaknath | 172.23±0.69 $^{a}$ | 1014.52±7.80 $^{a}$ | 1408.39±14.50 $^{a}$ | 1503.62±8.58 $^{a}$ | 39.15±0.49 $^{a}$ | 79.57±0.64 $^{a}$ | 47.08±0.11 $^{a}$ | 48.53±0.09 $^{a}$ |
| Crossbreed | 157.98±0.67 $^{b}$ | 1649.26±7.55 $^{b}$ | 1906.13±9.49 $^{b}$ | 1982.11±8.30 $^{b}$ | 62.47±0.47 $^{b}$ | 120.14±0.62 $^{b}$ | 49.47±0.10 $^{b}$ | 50.86±0.09 $^{b}$ |
| Genotype | NS | NS | NS | NS | NS | NS | NS | NS |
| AA | 165.00±0.76 | 1327.73±8.60 | 1655.15±10.81 | 1735.18±9.45 | 51.16±0.54 | 100.36±0.71 | 48.12±0.12 | 49.63±0.01 |
| AB | 165.00±0.76 | 1324.28±8.64 | 1655.16±10.86 | 1741.50±9.50 | 50.96±0.54 | 100.15±0.11 | 48.27±0.12 | 49.69±0.01 |
| BB | 165.33±0.95 | 1343.64±10.80 | 1661.47±13.58 | 1751.92±11.88 | 50.29± 0.67 | 99.06± 0.89 | 48.42± 0.15 | 49.77±0.13 |
| Breed × genotype | NS | NS | NS | NS | NS | NS | NS | NS |
| Kadaknath × AA | 171.33±1.03 $^{a}$ | 999.39±11.65 $^{a}$ | 1394.24±14.65 $^{a}$ | 1493.93±12.81 $^{a}$ | 40.72±0.73 $^{a}$ | 80.27±0.96 $^{a}$ | 46.73±0.16 $^{a}$ | 48.28±0.14 $^{a}$ |
| Kadaknath × AB | 172.03±1.08 $^{a}$ | 1010.03±12.22 $^{a}$ | 1418.00±15.36 $^{a}$ | 1504.00±13.43 $^{a}$ | 38.90±0.76 $^{a}$ | 79.50±1.01 $^{a}$ | 47.11±0.17 $^{a}$ | 48.60±0.15 $^{a}$ |
| Kadaknath × BB | 173.35±1.44 $^{a}$ | 1034.11±16.23 $^{a}$ | 1412.94±20.41 $^{a}$ | 1512.94±17.84 $^{a}$ | 37.82±1.02 $^{a}$ | 78.94±1.34 $^{a}$ | 47.40±0.23 $^{a}$ | 48.72±0.20 $^{a}$ |
| Kadaknath crossbred × AA | 158.67±1.12 $^{b}$ | 1656.07±12.65 $^{b}$ | 1916.07±15.90 $^{b}$ | 1979.42±13.90 $^{b}$ | 61.60.±0.79 $^{b}$ | 120.46±1.40 $^{b}$ | 49.52±0.18 $^{b}$ | 50.98±0.16 $^{b}$ |
| Kadaknath crossbred × AB | 157.96±1.08 $^{b}$ | 1638.53±12.22 $^{b}$ | 1892.33±15.36 $^{b}$ | 1979.00±13.43 $^{b}$ | 63.03±1 0.76 $^{b}$ | 120.80±1.01 $^{b}$ | 49.43± 0.17 $^{b}$ | 50.78±0.15 $^{b}$ |
| Kadaknath crossbred × BB | 157.98±0.67 $^{b}$ | 1653.18±14.27 $^{b}$ | 1910.00±14.27 $^{b}$ | 1990.11±8.30 $^{b}$ | 62.47±0.89 $^{b}$ | 119.18±1.18 $^{b}$ | 49.45±0.02 $^{b}$ | 50.82±0.18 $^{b}$ |

**Highly significant (P<0.01); NS, nonsignificant and values with different superscript differed significantly.

at 20, 40 and 52 weeks, egg production and weight at 40 and 52 weeks. However, the AA genotype in Kadaknath and AB genotype in Kadaknath crossbred birds showed affinity towards earlier sexual maturity. For adult body weight BB genotype was superior at 20 and 52 weeks and AB genotype at 40 weeks of age in Kadaknath, and AA genotype was superior at 20, 40 and BB genotype at 52 weeks of age in Kadaknath crossbred birds. Higher egg production was recorded for AA and AB genotype at 40 and 52 weeks in Kadaknath and Kadaknath crossbred birds respectively. Higher egg weight was recorded for BB genotype and AA genotype at 40 and 52 weeks in Kadaknath and in Kadaknath crossbred birds respectively.

The studied population of Kadaknath crossbred birds showed deviation from Hardy Weinberg however Kadaknath population was in Hardy Weinberg equilibrium. The birds of AA genotype in Kadaknath and BB genotype in Kadaknath crossbred showed earlier sexual maturity. The body weight at 20, 40 and 52 weeks in Kadaknath and Kadaknath crossbred birds of BB genotypes was higher as compared to AA and AB. The egg production at 40 weeks of age for AA genotype in Kadaknath and AB genotype in Kadaknath crossbred birds was recorded higher as compared to other genotypes. The egg weight of AA genotype birds was more as compared to AB and BB genotypes at 40 and 52 weeks in Kadaknath and Kadaknath crossbred birds.

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